THE OCCURRENCE OF MELANOPHORES IN CERTAIN EXPERIMENTAL WOUNDS OF THE GOLDFISH (*CARASSIUS AURATUS*)[1]

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While studying in the goldfish the repair of experimental wounds, crushes, burns, and fractures, it became apparent that melanophores developed in the wounds a few days after the trauma and later degenerated and thus disappeared. Not alone did these melanophores occur directly at the site of the injury, but not infrequently in the corium of adjacent areas and even in remote cutaneous regions. In none of these places were black pigmented cells seen by a previous low-power microscopic examination of the living fishes used for the experiment, nor were melanophores of the corium noticeable by high magnification in sections of tissue removed from the region of the wound at the time of trauma. The appearance of pigmented cells at the very point of injury seemed to indicate a rôle of importance for melanophores of this fish, from the viewpoint that these cells functioned in the processes of repair and, not unlikely, in the mechanism of body defense.

As results of different experiments were found to be uniform, only a few are here reported in detail as illustrative.

Experiment 1. Goldfish, 8 cm. long from snout to base of tail, kept in still water tank, supplied by current of air. Temperature of water 78° F.

Oct. 28, 1930. Transverse incision was made with a cataract knife through a single ray of caudal fin, near the upper edge of middle part of this fin. Incision penetrated tissues over both surfaces of fractured ray.

Oct. 30. Overlying the ray near the fracture are a few scattered melanophores with irregular processes (Fig. 1). Tissues overlying the fracture are œdematous and difficult to photograph for this reason. There are a few small points of hemorrhage near the fractured fragments.

Oct. 31. A large number of melanophores, in places interlacing, surround the proximal and the distal fragment of the fractured ray as if to encapsulate the fragments (Fig. 2).

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Nov. 1. Active degeneration of melanophores has begun with pigment granules lying free in tissue spaces (Fig. 3).

Nov. 5. Degeneration of all melanophores in the region of the fracture, with many small pigment masses scattered throughout the field.

Nov. 8. Entire region of fracture, somewhat whitish and translucent, shows no more evidence of pigment.

In the following experiment multiple injuries were produced.

Experiment 2. Two goldfishes, 7 cm. from snout to base of tail were placed in a tank of still water fed with a current of air. The temperature of the water was gradually raised from 70° F. by heating over a period of three days to 84° F.

Sept. 23, 1930. In both fishes eight different regions were clamped with an artery forceps each for 15 seconds. The points clamped were as follows: right and left operculum, both pectoral, both ventral, the anal and the caudal fins.

Sept. 25, 2 P.M. One fish shows early pigmentation by melanophores in caudal fin, the second fish has melanophores in the right ventral fin. Pigmentation is slightly distal to crush.

Sept. 26, 3 P.M. Three days after trauma, both fishes show pigmentation by melanophores at all eight points crushed. The pigmentation is a marked one due to the large number of melanophores present in the crushed zones and neighboring tissue.

Explanation of Plate 1

Figs. 1–4. Experimental linear fracture by incision of a ray of caudal fin of goldfish, the injury including all tissues directly overlying fracture. Letters *A* and *B* indicate site of fracture. All photomicrographs taken from the same living fish anæsthetised with chloretone 1–2000. Magnification × 90. Temperature of water 78°–80° F.

Fig. 1. Two days after injury. A few melanophores have appeared in the œdematous tissue near the fracture, *A.B.*

Fig. 2. Three days after injury. Numerous melanophores appearing as single cells or interlacing cells at the line of fracture, *A.B.*

Fig. 3. Four days after injury. Degeneration of melanophores at the site of fracture *A.B.* has begun. Small black pigment masses from degenerated cells lie scattered among living melanophores.

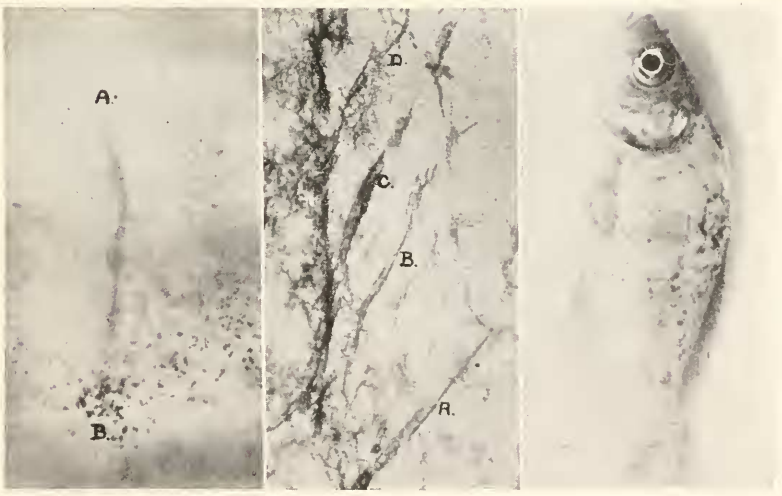
Fig. 4. Five days after injury. Degeneration of melanophores at the site of fracture *A.B.* is complete, scattered pigment debris remains in the field. Final disappearance of all pigment on the eighth day after injury.

Fig. 5. Inter-radial tissue of caudal fin showing melanophores distributed near capillaries marked *A*, *B*, *C*, *D*. × 69. Fresh tissue removed from goldfish near an area crushed eight days previously. Fish outdoors exposed to sunlight.

Fig. 6. Irregular areas of pigmentation of melanophores developing on the surface of the body of a goldfish injured by removal of all body scales five days previously. Photograph made from living fish anæsthetised with chloretone. Size, two-thirds normal.

PLATE I

1. 2. 3.



4. 5. 6.

Sept. 28. There is evidence of degeneration of melanophores at all crushed points. Temperature, 90° F.

Oct. 5. One fish is entirely clear of degenerated pigment granules at crushed point. The second shows a few black granules in the wound of the caudal fin.

Oct. 6. In both fishes all evidence of pigment formed of degenerated melanophores has disappeared at all eight points crushed. Thus these two fishes injured by crushing at eight separate points have shown, with temperature of water between 84° F. and 90° F., an intense pigmentation by melanophores at crushed points, a subsequent degeneration of melanophores, and a complete disappearance of all pigment detritus all in the course of 13 days.

Experiment 3. In this experiment, involving injury to the right operculum, 30 goldfishes, about seven cm. in length, were used. These were divided into three groups of ten. Each group was placed in a separate tank of running water in the laboratory. Fishes in Tank 1 were operated on by resecting one third of the right operculum by a straight vertical cut with scissors. Fishes in Tank 2 received a simple vertical crush for fifteen seconds of the middle of the right operculum. Fishes in Tank 3 were first crushed for 15 seconds by a clamp placed vertically in the mid-point of the operculum and all opercular tissue distal to the clamp was resected. Tank 1—Fishes (simple excision of one half of the right operculum) showed melanophores in the margin of the wound three days after operation. At first only a few such cells, but in the following two or three days there were many present. Evidence of degeneration of melanophores was noted in places as early as two days after their first appearance. Complete disappearance of black degenerated pigment from the wounded area varied between 3 and 9 days. Fishes in Tanks 2 and 3 with more severe injuries of the operculum showed a beginning accumulation of melanophores in the injured operculum also three days after trauma. The entire disappearance of pigment from the wound in fishes in Tank 2 (vertical crush of operculum) varied between 6 to 15 days after appearance of melanophores. In Tank 3 (fishes with crushed and partially resected right operculum) the eruption of melanophores at the injury occurred also three days after injury, but the final disappearance of pigmented debris varied between 9 and 16 days. One fish in Tank 1 and four fishes in Tank 2 showed slight pigmentation by melanophores of the opposite uninjured operculum, arising when the accumulation of melanophores on the injured side was well developed. Melanophores in the area of secondary pigmentation degenerated and disappeared before those in the experimentally injured right operculum.

Summary

The onset of cutaneous pigmentation by melanophores in three different types of wound of the operculum carried on simultaneously in three different tanks of running water at 76° F. was uniformly between the third and fourth day after trauma. The final disappearance of pigment of degenerated melanophores of the wound area varied between 6 and 19 days after injury. In some fishes the accumulation of melanophores noted at the wound was relatively slight; in others the black pigmentation caused by large numbers of melanophores was intense and remained over a longer period.

Fishes operated on during the cold winter months and kept in tanks of cold running water (43° F.) did not show at wounded areas such a rapid development of melanophores as described in the preceding experiment. Further, pigmentation of wounds under winter temperature extended over longer periods. Thus, in nine fishes with right operculum crushed for 15 seconds with an artery clamp placed at the middle of the operculum, followed by excision of opercular tissue distal to the operculum, the following results were obtained: An eruption at the injured operculum in all nine fishes occurred between 13 to 16 days after injury; pigmentation had cleared up by degeneration of melanophores in only three fishes two months after injury, with temperature of water at 53° F. It took approximately one more month (temperature 53°–56° F.) for four more fishes to clear; the remaining two fishes cleared at the end of still another month or four months from the date of injury, when the temperature of the water had gradually risen to 61° F. The longest period of pigmentation in a wound of this series represented approximately 110 days from the date of the first appearance of melanophores.

It became of interest to learn whether or not in fishes kept in very cold water, an appearance of melanophores after trauma could be temporarily inhibited, to appear for the first time when such fishes were changed back slowly to more favorable warmer temperatures. A number of experiments were done along these lines.

Experiment 4. A goldfish, seven cm. in length, was placed in a tank of still cold water supplied by a current of air, the water varying in temperature between 42° F. and 45° F. The tank was set up in a refrigerator arranged with a double window, admitting ample daylight. It was found advisable to accustom the experimental fishes gradually to cold. By using several submerged electric lights at the beginning of the experiment and turning these off as desired, the temperature of the water could be lowered slowly without endangering the life of the fish.

Oct. 14, 1930. A small incision was made with a cataract knife in the caudal fin of this goldfish dividing transversely a single ray near the upper margin of the fin. Examination of melanophores at four day intervals negative for an entire month. Temperature 42° to 46° F.

Nov. 14. Temperature in tank raised slowly so as to reach 66° F. on Nov. 16th.

Nov. 16. Numerous melanophores appeared for the first time at fracture and along injured ray distal to this. No other black pigmentation noted.

Nov. 25. Slight pigmentation by melanophores of tip of tail and also along the margin of dorsal fin. Large accumulation of melanophores at fracture.

Nov. 27. Active degeneration of melanophores at fracture and other pigmented regions.

Nov. 29. Fish under dissecting microscope shows no pigment masses either at site of experimentally fractured ray or at the secondary points of black pigmentation of tail or dorsal fin. All melanophores have disappeared by a process of degeneration.

Fishes kept in a dark chamber, excluding all light, developed melanophores in wounds as promptly as did controls kept in daylight.

Experiment 5. Two goldfishes, seven cm. in length, with crushed right operculum and caudal fin, kept in a dark chamber in a tank of still water at 64° F., supplied by air current, were taken out of this chamber to be examined for the first time after injury on the fifth day. Many melanophores were present in crushed regions. At the same time, two control fishes, injured on the same day in a similar way, kept in a tank of equal size at the same temperature but exposed to laboratory daylight, exhibited, also for the first time, a large number of melanophores at the two crushed points. Twenty-three days after injury, one fish contained in the dark chamber and both controls were clear of pigment; the second fish in the dark chamber showed no melanophores in the injured operculum, although a few small masses of degenerated pigment masses still remained in the caudal fin.

The production of a second injury in a healed wound frequently, but not always, caused another eruption of melanophores. Refracturing a single ray at the same point, especially where the previous healing had left a wide whitish translucent area, did not produce a second crop of melanophores. The very simple injury of making a longitudinal slit in the caudal fin did not call forth melanophores either at the time of the first injury or with repeated incisions at the same point.

The irregular topographic distribution of melanophores following

trauma was seen particularly well in experiments where the scales on both sides of the body were totally removed.

Experiment 6. Nov. 1930. Three goldfishes, *A*, *B*, *C*, measuring 8, 7, 5 cm. in length respectively, kept in a heated tank of still water 76° F., supplied by air current, were operated on under chloretone anæsthesia (1–2000). All scales of the body were removed with forceps in all three fishes.

Dec. Four days after operation, melanophores appeared in irregular groups at various points on both sides of the body. The two larger fishes, *A* and *B*, showed in the course of the next few days a large number of melanophores in irregular scattered patches. The patches of pigmentation by melanophores in fish *B* are shown in Fig. 6. The smallest fish, *C*, showed only a few melanophores in small, widely-scattered areas. By the end of the twelfth day degeneration of melanophores evoked by removal of scales had occurred in all three fishes with a disappearance of broken-down pigment material. At this time (12 days after removal of scales) each fish showed definitely a set of new young scales. Fish *B* successfully withstood a second complete removal of scales, under chloretone anæsthesia, but this time only a very few rapidly degenerating melanophores developed on the denuded surface of the body, as if the supply of pigment-forming cells for these particular surface areas were partially exhausted. When, however, on the fourth day after the second operation for removal of scales the caudal fin of this fish was crushed by clamp for 15 seconds, numerous melanophores developed three days later in the crushed tail but in no other place.

Discussion and Summary

Various important problems relating to melanophores and melanogenesis appear in connection with the works of Van Rynberk (1906), von Frisch (1911), Weidenreich (1912), Asvadourova (1913), Spaeth (1913), R. Fuchs (1914), Wyman (1924), Wells (1925), Abolin (1925), Ewing (1926), Jost (1926), Bloch (1927), Cordier (1928), Becker (1930).

For the present purpose it may be of interest to recall that a number of years ago Weidenreich (1912) showed that in vertebrates the distribution of black pigment cells could be regarded as forming four distinct envelopes for the body. These envelopes he designated as "cutaneous, perineural, pericoelomatic and perivascular" respectively. He pointed out that whereas in some vertebrates several or all of these pigmentary envelopes were well developed, in other vertebrates one or more of these pigmentary envelopes might be found poorly developed, showing only a trace or rudiment of pigmented tissue. For example,

in man, where there exists a well developed cutaneous envelope of pigmented tissue, the perineural pigmented tissue is poorly developed, presenting itself as scattered black pigment cells of the piamater and elsewhere in the brain. In fishes all pigmentary envelopes are regarded as fairly well developed.

In interpreting the meaning of melanophores following injury as seen in the above experiments on goldfish, it should be kept in mind that such melanophores may represent a perivascular or perineural type of cell developing the properties of forming pigment, rather than cells belonging strictly to a system of cutaneous melanophores. It is particularly the cutaneous or corial melanophores of fishes which have received the most study to date.

Melanophores, according to Bloch (1927) show a number of morphologic peculiarities in that they form processes or dendrites and have a tendency to arrange themselves in an interlacing network. They exhibit in cold-blooded animals certain functional reactions which are shown by the spreading or the contraction of the intracellular masses of pigment granules. These reactions are changes which have their origin in nervous, actinic or hormonal stimuli; and they may also be produced by mechanical, chemical and electric means.

Ever since the description of melanophores in fishes by Siebold (1861), many investigators have contributed to the morphology of this subject. The works of Ballowitz (1912–16) on the different types of chromatophores (*i.e.*, the melanophores, xantho or erythrophores, guanophores and their various combinations forming what he designated as chromatic organs) have largely laid the basis for our present knowledge of pigment cells in fishes. This author also demonstrated histologically the innervation of melanophores in fishes.

The experimental observations of Pouchet (1876) showed a relationship between cutaneous melanophores in fishes and the sympathetic nervous system. It remained, however, for von Frisch (1911, 1912), in a series of important experiments, to demonstrate in fishes a contraction center for cutaneous melanophores in the front part of the medulla, and a secondary center in the spinal cord. Further, he explained the pathways by which impulses pass from brain through pigment motor nerve fibers to the sympathetic system and from here by means of the peripheral nerves not only to the melanophores but also to other chromatophores of the skin.

In general, the function of melanophores has been variously interpreted. In addition to the view that cutaneous pigmentation and pigment changes represent color adaptation to environment, the purpose of cutaneous pigment has been thought to lie in its protection of

deeper tissues against injurious solar rays. The migration of retinal pigment granules as it applies to vertebrates and arthropods is thought by Parker (1906) to be a mechanism calculated to protect the receptive organs of the retina from over-stimulation by light and to improve the retinal images. Cutaneous pigment cells have been regarded as transforming light into heat energy. According to this view, as Weidenreich (1912) explains, the minute individual intracellular pigment granules of melanophores become heat bodies or Heizkörper, which distribute heat to neighboring protoplasm. Weidenreich (1912) has further suggested, because melanophores are innervated and react to optic, thermic and chromatic stimuli, that they may be regarded perhaps as sensory cells for color and warmth perception.

Cordier (1928) believes that the formation in cells of melanin is a process of excretion as yet not well understood. The theory implies that certain toxic waste products of metabolism gain access to special cells and there become insoluble and pigmented, their toxic products being neutralized. Elimination of pigment follows slowly as if it were a process of retarded excretion. Certain clinical cases of Addison's disease and melanosarcoma have shown melanin greatly increased in cutaneous areas and present in the blood and in the urine. This has been taken to mean a profound chemical disturbance of the body as a whole and gives support to the view that a general metabolic process may ordinarily affect the production of melanin in various regions of the body.

Whatever may be the relationship to the nervous system of melanophores resulting from trauma as seen in the present experiments on goldfish, it seems plausible from their structural arrangement in healing wounds, that such melanophores are pigmented cells which function in repair of damaged tissue. Melanophores of this kind appeared relatively early in the course of wound-healing when favorable warm temperatures were employed. They disappeared by a process of degeneration at the site of the wound when healing proceeded and usually when the covering of the wounded surface was nearing its completion. Whereas melanophores showed in wounds of goldfishes within 3 or 4 days after injury when fishes were kept in water of relatively warm temperature (70°–90° F.), with fishes kept in cold water (40°–42° F.) the appearance of melanophores in wounds was retarded or even inhibited, to appear for the first time when these fishes were returned to a warm environment. A temperature of 40° F. was found sufficient to inhibit the appearance of melanophores for a month.

Fishes kept in a dark chamber completely excluding light showed melanophores in various experimental wounds as early as did controls

kept under usual laboratory conditions exposed to light. Fishes kept in tanks out-of-doors and in this way exposed directly to the sunlight developed melanophores in wounds a few days later (Fig. 5). The reaction here seemed intense. In some of these fishes melanophores developed not alone at the crushed points, but also in areas adjacent to the wound and in all other fins.

When studied in a simple form of injury such as dividing transversely a single ray of the caudal fin, melanophores appeared first as periadventitial cells in close relation to the outer walls of the small capillary blood vessels which covered the surface of the ray near the fracture. With an increase in numbers, the melanophores spread toward the region of the fracture and formed a network (Fig. 2) in the corium by the interlacing of the numerous irregular processes. Degeneration in individual melanophores was observed as early as 24 hours after their first appearance near a fractured ray. Fixed paraffin sections of tissue with degenerating melanophores showed a moderate number of phagocytic cells containing pigment. For the most part, however, the impression was gained that the pigment detritus rested free in the tissue spaces preparatory to removal by lymphatics, or became dissolved *in situ*.

The actual production of melanin in cells is now generally regarded as the result of enzyme action. The important studies of Bloch (1927), advancing the views on the intracellular production of melanin by enzyme, are too well known to need repetition here. It is conceivable that in the experimental wounds of goldfish chemical changes occur locally permitting melanin to be formed in periadventitial cells irregularly distributed in the corium of the injured area.

Experimental wounds of goldfishes quite naturally are constantly open to infection by bacteria or parasites. Numerous bacteria and especially cocci were seen in paraffin sections of tissue from crushed operculum at various stages after injury before complete healing had occurred. When, as occasionally noted, a growth of fungus appeared in connection with experimental wounds, pigmentation by melanophores appeared particularly intense, affecting not alone the wound but also adjacent areas. There was at times pigmentation of the fins other than the ones experimentally injured and, in rare instances, a patchy pigmentation of body scales under these circumstances. Treating such wounds for several days in succession with two per cent mercurochrome destroyed the parasites, and pigmentation of the wound with secondary pigmented areas then disappeared. The presence of bacteria in wounds and the large number of melanophores present in injured areas affected with parasites, suggest a possible rôle for melanophores in the mechanism of body defense.

Goldfishes subjected to a total removal of scales showed in the course of several days a distribution of melanophores varying in extent and intensity in different fishes. This eruption was asymmetrical, irregular and patchy, as if periadventitial cells capable of forming black pigment as a result of trauma or during subsequent wound regeneration, actually occupied a very irregular distribution on both sides of the body. As new scales formed in these experimentally produced scaleless fishes, melanophores disappeared by degeneration. A second total removal of scales in one of the fishes was followed by a very scanty eruption of melanophores, as if the possibility of local melanophore production in this instance were, temporarily, at least, exhausted.

Usually, but not always, a re-injury at the same point brought out a second eruption of melanophores differing but little from that which followed the primary injury.

The eruption of melanophores in experimental wounds of the goldfishes, varying in intensity in different fishes, appears to indicate that such melanophores, probably periadventitial in origin, form in response to injury and function in the repair of injured tissues.

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